The evolution of phototransduction from an ancestral cyclic nucleotide gated pathway

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The evolutionary histories of complex traits are complicated because such traits are comprised of multiple integrated and interacting components, which may have different individual histories. Phylogenetic studies of complex trait evolution often do not take this into account, instead focusing only on the history of whole, integrated traits; for example, mapping eyes as simply present or absent through history. Using the biochemistry of animal vision as a model, we demonstrate how investigating the individual components of complex systems can aid in elucidating both the origins and diversification of such systems. Opsin-based phototransduction underlies all visual phenotypes in animals, using complex protein cascades that translate light information into changes in cyclic nucleotide gated (CNG) or canonical transient receptor potential (TRPC) ion-channel activity. Here we show that CNG ion channels play a role in cnidarian phototransduction. Transcripts of a CNG ion channel co-localize with opsin in specific cell types of the eyeless cnidarian Hydra magnipapillata. Further, the CNG inhibitor cis-diltiazem ablates a stereotypical photoresponse in the hydra. Our findings in the Cnidaria, the only non-bilaterian lineage to possess functional opsins, allow us to trace the history of CNG-based photosensitivity to the very origin of animal phototransduction. Our general analytical approach, based on explicit phylogenetic analysis of individual components, contrasts the deep evolutionary history of CNG-based phototransduction, today used in vertebrate vision, with the more recent assembly of TRPC-based systems that are common to protostome (e.g. fly and mollusc) vision.

Keywords: complex trait evolution; ancestral state; phototransduction; opsin; cnidarian; cyclic nucleotide gated ion channel

1. INTRODUCTION

Understanding the evolutionary origins of complexity is a central issue in biology. Studies of complex trait evolution are commonly conducted by mapping the presence or absence of whole, integrated traits on a phylogeny of related species. While such approaches allow important comparative inferences to be made on the timing and number of origins of the trait under investigation (Cunningham et al. 1998; Oakley & Cunningham 2002; Reznick et al. 2002; Avise 2006), they do not inform our understanding of the histories of its individual integrated components. Such individual components often vary over time and can shed light on the evolutionary patterns and processes of new trait origination (King et al. 2003; Edwards & Donoghue 2006; Plachetzki & Oakley 2007). Because the genes that specify individual components of complex traits are becoming increasingly well understood, hypotheses on the ancestral composition of such traits can now be tested by integrating studies on the evolution of their individual components.

Animal phototransduction cascades are prime examples of complex traits, with components that have been well characterized on functional, biochemical and genetic levels. Despite this progress, the evolutionary origins of these multi-component cascades remain little understood and have been the subject of both longstanding puzzlement among evolutionary biologists (Darwin 1859, 1887) and cynicism among others (Rehe 1996). In particular, two types of ion channel delineate different modes of phototransduction known in animals. First, cyclic nucleotide gated (CNG) ion channels function in a rhabdomeric pathway where activation leads to a depolarizing cell-physiological response to light (Matute & Zagotta 2003). Conversely, canonical transient receptor potential (TRPC) ion channels, members of a larger gene family of TRP channels (Venkatachalam & Montell 2007), function in a rhabdomeric pathway where activation leads to a hyperpolarizing cell-physiological response to light (Hardie & Raghu 2001). Both CNG- and TRPC-modulated pathways are initiated by class-specific opsin paralogues (c-opsin and r-opsin, respectively), which are both present in protostomes and deuterostomes, and therefore predate the origin of bilaterian animals (Arendt et al. 2004; Velarde et al. 2005). In vertebrates, primary vision is mediated by CNG-based ciliary phototransduction, whereas in protostomes a TRPC-based rhabdomeric cascade is thought to serve this role (Hardie & Raghu 2001). Despite the centrality of these signalling pathways to vision and other photosensitivity phenotypes, an understanding of the origins of these cascades in animal evolution has evaded biologists, largely because accumulation of data from...
non-bilaterian animals like Cnidaria has lagged far behind the availability of data from bilaterians—especially flies, molluscs and vertebrates.

Therefore, as the only non-bilaterian extant animal lineage known to possess phototransductive opsins proteins (Plachetzk et al. 2007; Suga et al. 2008), data from cnidarian taxa such as anemones, jellyfish and hydra can inform our understanding of the origin and early evolution of phototransduction. Elements of the phototransduction machinery that are shared between cnidarians and bilaterian animals may have been present at the origin of opsins-based photosensitivity phenotypes in animals. Here, we integrate studies of phylogenetics, gene expression, behavioural pharmacogenetics and character evolution to show that a CNG-based phototransduction system was the ancestral state of opsin-mediated photosensitivity and visual phenotypes in animal evolution. Our results highlight the ancient assembly of CNG-based ciliary phototransduction, which underlies function in vertebrate rods and cones, and the evolutionarily more recent assembly of the TRPCl-based visual pathways that are common to the rhabdomeric photoreceptors of insects and other protostomes.

2. MATERIAL AND METHODS

(a) Data mining

Publicly available gene models for all but the sponge Amphimedon queenslandica were compiled using BLASTDB and subjected to TBLASTN (Altschul et al. 1997) searches using a wide range of bilaterian CNG and TRP loci as bait. For A. queenslandica, trace genome data were mined (http://www.ncbi.nlm.nih.gov/Traces).

For opsins phylogeny, we first obtained opsin sequences that could be specifically correlated with either CNG, TRP or, in the case of photoisomerases, no phototransductive activity (electronic supplementary material, table S3). These were then used as bait sequences in similarity searches using BLASTP (Altschul et al. 1997) of non-redundant protein databases curated by uniprot (http://www.uniprot.org). For each opsin where the phototransductive ion channel was known, an additional two sequences from uniref90 and two sequences from uniref50 were retained. Redundant sequences obtained by this procedure were removed (see electronic supplementary material).

(b) Phylogenetic analyses

Amino acid sequences comprising CNG, TRP and opsin datasets were aligned under default parameters using TCOFFEE (Notredame et al. 2000) and alignment manipulations were done using Salign (Galtier et al. 1996). Phylogenetic analyses were conducted using maximum likelihood (ML) as implemented in RAxML (Stamatakis et al. 2005) and Bayesian Markov Chain Monte Carlo (BMCMC) approaches using PhyloBayes (Lartillot & Philippe 2004). Support for internal nodes was assessed with 1000 bootstrap replicates for ML, and posterior probability for BMCMC analyses. ML analysis assumed the best-fit model WAG + I + G determined by ProtTest (Abascal et al. 2005). BMCMC analyses were conducted under the default CAT approach. Convergence of BMCMC chains was determined to have occurred once the mean likelihood difference between chains fell below 0.01. Bayesian ancestral state reconstructions were done in SIMMAP using stochastic mutational mapping (Bollback 2006) and 20 000 trees sampled from the posterior (the consensus of which is given in electronic supplementary material, figure S6). Character states were reconstructed under a discrete three-state model with equal priors on character transition bias, and using branch length as rate. MESQUITE (Maddison & Maddison 2004) was used for ancestral state reconstructions under ML.

(c) In situ hybridization

A partial transcript for hmCNG was cloned by reference to an expressed sequence tag (GenBank accession no. DT606755). The isolation of the hmOps2 clone has been described previously (Plachetzki et al. 2007). The expression of hmOps2 and hmCNG was studied using multi-channel in situ hybridization. RNA probes were synthesized using DIG (hmOps2) and fluorescein (hmCNG; Roche). Hybridization was conducted as described by Greens et al. (1995), with modifications, and co-localization data were collected using a Fluoview 500 confocal microscope (see electronic supplementary material, methods).

(d) Animal culture and photobehaviour assay

Cultures of Hydra magnipapillata were maintained using standard methods (Lenhoff 1982). Individual hydras were tested in 5 ml of hydra medium in 20 ml pyrex dishes. Subjects were staged for a minimum of 20 min in total darkness to allow dark-adaptation and recovery from any transportation shock. After 20 min, individual subjects were exposed to blue light of either 5 lux for dim light trials or 3500 lux for bright light trials. Light of a sharply defined spectrum peaking at 470 nm was emitted from a light-emitting diode (LED) array (SuperBright LEDs). Light intensity as given in lux was measured using a Smart Luxmeter (Aquatic Ecosystems, Inc.). Hydra were tested for the contraction response (Passano & McCullough 1962, 1965) in individual arenas so that only one individual was tested at a time. The time until first complete contraction was recorded and experiments were terminated after 20 min if the animal did not contract. For pharmacological treatments, cili-dilazem was used at a concentration of 1 μM in standard hydra medium (see also electronic supplementary material, methods).

3. RESULTS

We first identified homologues of ion channel genes, with particular focus on non-bilaterian genomes. Bioinformatic screens recovered loci belonging to both the CNG and TRPC ion-channel gene families from most of 22 animal genomes included in the study, with the exception that TRPC loci were not recovered from the genomes of the sponge A. queenslandica or the placozoan Trichoplax adhaerens (a full listing is given in the electronic supplementary material, tables S1 and S2). A single CNG locus (hmCNG) with membership in the CNG A clade, which also includes representatives that function in vertebrate phototransduction, was recovered from the genome sequence of the hydra (electronic supplementary material, figures S1 and S2). A single putative but truncated TRPC locus was also identified in the hydra genome, but this gene is neither found in the hundreds of thousands of publicly available hydra EST sequences, nor could it be amplified from our cDNA preps. In addition,
Figure 1. Studies of gene expression and behavioural pharmacogenetics suggest the involvement of a CNG ion channel in cnidarian phototransduction. (a–c) Transcripts of cnidarian opsin and CNG ion channels co-localize to the same cell types. (a) hmOps2, red; (b) hmCNG, green; (c) merge; nuclei, blue. Confocal optical section = 0.2 μm. Scale bar = 50 μm. (d) The contraction response (top) in the hydra is a photobehaviour that is perturbed by a CNG inhibitor but not a TRPC inhibitor. Animal subjects were adapted in total darkness prior to presentation with bright light (see §2). For each treatment, behavioural trials were conducted under conditions of blue light (3500 lux, 470 nm; white bars) or dim conditions (5 lux, 470 nm; black bars). Under control conditions a significant difference exists in the behaviour of animal subjects, indicating a direct response to light. Under treatment with the CNG inhibitor cis-diltiazem (5 μM), the frequency of the response to bright light is decreased significantly. However, no significant difference exists between bright light trials conducted in the presence of the TRPC inhibitor SKF 96395 (1 μM). Animals treated with either drug were responsive to mechanical stimuli following trials. Drugs were used at the highest non-lethal concentrations. Contraction response illustration is from Passano & McCullough (1963).
Figure 2. Ancestral state reconstruction supports the hypothesis that CNG ion channels functioned in the ancestral phototransduction cascade. A phylogeny for animal opsins represents the evolutionary history of the phototransduction cascades. Outgroups (grey) were selected based on a previous study of GPCR evolution (Fredriksson et al. 2003). Functional relationships between specific opsin sequences and their ion channel types were coded based on new data reported here and those abstracted from the literature (orange, CNG; blue, TRPC; black, no phototransduction as per photoisomerase). See electronic supplementary material, table S2 for justification of functional data. Additional sequences (white) mined from public databases (see §2). Reconstruction of ancestral states using character mapping reveals, with a strong proportion of likelihood (pie chart), that the ancestral animal phototransduction cascade used a CNG ion channel (orange; proportion of likelihood 0.99). The node joining the rhabdomeric opsin is also reconstructed, with a high proportion of likelihood, to have used a TRPC ion channel (blue; proportion of likelihood = 0.99). Additional analyses using Bayesian methodology strongly support these findings. Outgroup rooted ML tree is shown (see electronic supplementary material, figure S5 for support indices). Posterior probabilities (PP) of reconstructed states from Bayesian analyses integrate over all topologies sampled from the posterior (electronic supplementary material, figure S6) and allow multiple character state transitions to occur along branches.
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Figure 3. Examining the histories of their individual components can illuminate the origination of complex traits. (a) Illustrated is a traditional view of animal phylogeny (Philippe et al. 2009) with the presence (grey) and absence (white) of opsin-based phototransduction mapped onto the tree using parsimony. This approach illustrates that cnidarians, including Hydra magnipapillata, are in a key phylogenetic position to inform our view of the origins of this complex trait. (b) Analysing the individual components of phototransduction in a phylogenetic context yields deeper insights into its origin and evolution. Round-cornered rectangles represent phototransduction (grey) or other G-protein-coupled receptor (GPCR) pathways (white), with individual components inside. Our results indicate that CNG ion channels are involved in hydra phototransduction (figure 1) as well as the ancestral phototransduction cascade (figure 2). Because CNG is present outside cnidarians and is involved in non-opsin GPCR pathways (Stumpf et al. 2009), we infer that—like G-proteins and other phototransduction components (Suga et al. 1999; Plachetzki et al. 2007)—CNG ion channels predate phototransduction. Therefore, the origin of phototransduction may have involved the gain of light sensitivity in an ancient CNG–GPCR pathway. Our results also indicate that TRPC-based rhabdomeric phototransduction originated by changing multiple components. Protostomes and deuterostomes are illustrated to have both ciliary and rhabdomeric pathways (Arendt 2003), with the pathway dominant in vision positioned on top. Dashed lines indicate major clades in metazoan evolution. Additional abbreviations: c-opsin, ciliary opsin; r-opsin, rhabdomeric opsin; I, intermediary molecules; Ic, ciliary intermediaries such as phosphodiesterase (PDE) and guanylate cyclase (GC); Ir, rhabdomeric intermediaries such as phospholipase C (PLC), DAG (diacylglycerol) and PIP2 (phosphatidylinositol-4,5-biphosphate).

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used in the rhabdomeric visual systems of many modern invertebrates probably originated from within a CNG-based radiation of cascades. This transition probably involved an exchange of multiple components because the transition we report here, from CNG to TRPC ion channel, is also associated with a change to Gα-q as the G-protein component of rhabdomeric phototransduction (Hardie & Raghu 2001). Such a G-protein transition, occurring prior to the bilaterian ancestor, may have been caused by amino acid changes in the third cytoplasmic loop of opsin, which activate Gα-q (Marin et al. 2000; Arendt et al. 2004; Plachetzki et al. 2007) and lead to modulation of TRPC ion channels. While we infer that this transition gave rise to a novel light-gated cell physiology, which also correlates temporally with the origin of a new morphological class of photoreceptors (Eakin 1979; Arendt 2003), other alterations in Gα subunit usage that did not precipitate shifts in ion channel usage must have also occurred. Our previous attempts to reconstruct the ancestral G-protein complement in animal phototransduction proved equivocal (Plachetzki et al. 2007), because as many as four different Gα paralogues are presently known to participate in various modes of animal vision, including Gαi in vertebrates (Franke et al. 1990), Gαo in molluscs (Gomez & Nasi 2000), Gα in cubozoan cnidarians (Koyanagi et al. 2008) and Gαq in many protostomes (Hardie & Raghu 2001). While our current analyses suggest that the ion channel components of animal phototransduction cascades remain deeply conserved in evolution, the Gα components of these systems appear to be less conserved.

Although the origin of rhabdomeric phototransduction involved multiple components (including TRPC, Gαq and other intermediates associated with this pathway; see figure 3b), the origin of phototransduction in animals may have involved the alteration of only a single component—the receptor—allowing gain of light sensitivity in an ancient CNG-based GPCR pathway. We infer that the CNG ion channel component of the ancestral phototransduction pathway was present prior to the origin of animal opsins. CNG loci were recovered from taxa outside Eumetazoa (electronic supplementary material, figures S1 and S2) and, based on comparison of opsin-mediated pathways with related outgroup GPCR pathways like human melatonin (Stumpf et al. 2009), probably functioned in other GPCR pathways prior to the origin of functional opsins. In addition, earlier studies showed that G-proteins and other components of animal phototransduction cascades diversified prior to Metazoa (Suga et al. 1999). Of the components studied thus far, only the origin of opsin from non-opsin GPCR genes (by definition) corresponds with the origin of animal phototransduction (Plachetzki & Oakley 2007). Animal opsins originated prior to Eumetazoa, but opsins are unknown
from non-animals, including fungi and choanoflagellates, and non-Eumetazoan animal lineages, such as poriferans and placozoans (Plachetzki et al. 2007; Suga et al. 2008). Further, type-I opsins common to bacteria and other taxa are not homologous to animal opsins (Spudich et al. 2000; Larusso et al. 2008).

Taken together, these data support a view that the origin of animal phototransduction, and by extension of animal vision itself, involved the evolution of an existing CNG-based cascade into a functionally new cascade, founded by a novel opsin lineage of light-sensitive G-protein-coupled receptors. Our inferences that CNG and G-proteins predate animals are robust to alternative views of animal phylogeny, but the specific timing of opsin origin depends on these assumptions. If the traditional view that sponges are the sister group to other animals is assumed (Philippe et al. 2009), then the functional transition to light sensitivity occurred during the phylogenetic interval separating Eumetazoa from earlier animal lineages at least 600 Myr ago (Peterson et al. 2004). A second major transition, from CNG- to TRPC-based phototransduction, would have occurred much later in animal evolution predating the bilaterian clade and giving rise to the rhabdometric system.

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